THE KANSAS UNIVERSITY SCIENCE BULLETIN.

Vol. IX, No. 8—December, 1914.

(Whole Series, Vol. XIX, No. 8.)

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PUBLISHED BY THE UNIVERSITY,

LAWRENCE, KAN.

Entered at the post-office in Lawrence as second-class matter.

5-2496

KANSAS STATE PRINTING OFFICE.
W. C. Austin, State Printer.
TOPEKA. 1915.

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VOL. IX, No. 8.] DECEMBER, 1914. [WHOLE SERIES VOL. XIX, No. 8

Some Methods of Embryological Technique.

BENNET M. ALLEN.

Contribution from the Zoölogical Laboratory No. 210.

THERE are two distinct lines of work to be outlined in this paper. These are of especial value not only in research work, but in the preparation of material for laboratory instruction.

The writer has long felt that there should be some satisfactory method of preserving fine dissections, free-hand sections, opaque eggs, etc., in such a manner that they could be studied by reflected light under the low power of the microscope. It has become the almost universal practice to clear an object and to mount in some such substance as Canada balsam, glycerine jelly, etc. The study of small opaque objects has been too greatly neglected.

Experiments were made in imbedding entire frog eggs. At first glycerine jelly was used, but this was found to be altogether unsatisfactory owing to its clearing properties. This is precisely the thing that we wish to avoid. So gelatine alone was employed with very satisfactory results. This is made up as follows: First of all thymol was dissolved in distilled water to saturation. In accomplishing this heat is employed. If the solution becomes murky upon cooling, it is filtered until clear. The gelatine should be prepared in the usual way—that is, it should be allowed to absorb as much as possible of this thymol water, and the excess water is drained off. Then it is ready to be heated and used.

Received for publication October 28, 1914.

The gelatine solution is kept in test tubes that can be corked, and when desired for use these are placed in a water bath. The liquefied gelatine is then poured out on the slide and allowed to harden for a few minutes. In order to make cells suitable for mounting large objects, strips of glass are pressed upon a slide in a manner indicated in the diagram. The

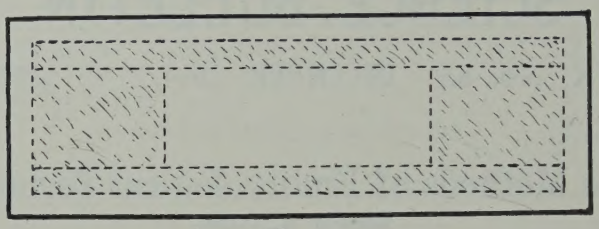

Fig. 1

liquefied gelatine is poured into the space in the center. Within a few minutes after solidification, a hot needle is used to melt small points in the gelatine into which the objects to be mounted are then inserted. In this manner they can be placed in any arrangement desired, and held in position with the needle until the surrounding gelatine solidifies.

It is thus possible to mount as many as fifteen or twenty properly arranged frog eggs in different stages of segmentation. Care should be exercised to have the gelatine deep enough in the cell to almost fill it up. After allowing the gelatine to cool to such a degree that all of the objects are held firmly in place, gelatine heated barely enough to have a liquid consistency, is then poured into the cell in sufficient quantity to flood it, and a slightly warmed slide is placed upon the cell in such a manner as to coincide in position with the slide below. In preparing it, care must be exercised to prevent the inclusion of air bubbles in the cell, and at the same time furnish a complete film of melted gelatine between the cover slide and the underlying glass strips. This slide should be held in place until the gelatine has solidified, thus holding it firmly in position.

It is next necessary to seal the mount. This is done by first cleaning out the excess gelatine from the grooves formed by the strips between the margins of the two slides and the edges of the strips between them. This can be done with a small piece of wood like a toothpick. Care should be taken to clean away the gelatine quite thoroughly. It is even advisable to cleanse

the groove with a moist cloth or camel's hair brush. It should then be thoroughly dried in like manner. All of this requires care and patience, and the permanency of the mount depends upon the neatness with which it is done.

Then some cement, such as gold size or asphaltum, is run into the groove along all four sides, and the preparation is set aside for the cement to harden. From day to day additional cement is added, until by its successful solidification the groove is completely filled up. The preparation should then be permanent, with the precaution that it should not be subjected to heat such as might be caused by placing it where direct sunlight will fall upon it. The gelatine can be hardened by the simple process of previously infiltrating the objects to be mounted with a solution of formalin. After mounting, this will gradually diffuse out into the gelatine and harden it.

This method can be of quite wide application. It could be used in free-hand sections, for instance, one can cut in half the blastulæ and gastrulæ of the frog. It has always been a problem to properly preserve the halves of such gastrulæ that one obtains by cutting the egg in two with a safety-razor blade. Preparations showing various stages of gastrulation were mounted in the manner described, and proved very instructive. Embryological or other objects might well be cut into thick opaque free-hand sections. These should prove extremely instructive, opening up new and very important fields of embryological work.

Various modifications of the foregoing method may be used. It is quite possible to use hollow-ground slides designed for use with a hanging drop. In such cases as flooding the cell at the completion of mounting, one can use a circular cover slip to cover it, and this can be ringed on a turntable in the usual manner. One must exercise great precaution to avoid overheating the gelatine. When a needle is used to heat a place for the insertion of one of these objects, one should give a little time to allow the gelatine to almost solidify, the object can then be placed in position and blown upon, or the slide placed upon ice so that solidification may be greatly hastened. In this way it is often possible to imbed rather delicate objects with very large cavities, such as the medullary fold stages of Ambystoma.

In making preparations of this kind, where deeply pigmented specimens are used, it is often found desirable to bleach them. Beautiful preparations of the segmentation stages of the frog can be made by the methods explained above in which the material has been prepared by immersion in hydrogen peroxide—the ordinary commercial strength can be used. It is only applicable with specimens that have been thoroughly hardened in any of the ordinary hardening fluids, or even in formalin. In the latter case care must be taken with delicate structures to wash out the formalin before putting them in the hydrogen peroxide, otherwise the tissues would be distorted by the oxygen liberated. Frog eggs may be left in the hydrogen peroxide for even two or three weeks, if necessary. My experience has been that about a week will suffice for the bleaching of the pigmented area to a light brown color.

When this has been accomplished to the right stage, the segmentation furrows show with remarkable clearness. One great difficulty in using the frog as a type form for a study of elementary embryology has been the fact that it has appeared impossible to make whole mounts of the young tadpoles. Well-fixed specimens in all stages of development can be completely bleached with hydrogen peroxide and rendered as white as chick embryos. They can then be stained and mounted in balsam. My experience has been that a very dilute stain of alum cochineal gives the best results, although I have also used a dilute stain of Conklin's hæmatoxylin. Emphasis must be laid upon the dilution of these stains. In the case of both, they are diluted with distilled water until they show but a faint tint of color.

The tadpole is left in the stain over night, and is then run up through the increasing grades of alcohol, being finally transferred into one of the better clearing oils such as creosote or synthetic oil of wintergreen. The tadpole is then mounted in Canada balsam or dammar.

These preparations have a very great advantage over chick embryos, in the fact that no parts are missing; there is no distortion due to imperfect fixation, and there is no torsion of the body. If the preparation is properly carried out, they should be just as clear and as easily studied as are whole mounts of the chick. The brain, pituitary ingrowth, eye, auditory vesicle, pronephros, heart, stomodæum and notocord

can be made out with great clearness. It is even possible to see the outlines of the cranial and spinal nerve roots.

Experience in teaching by means of these preparations has demonstrated the ease with which they can be studied and understood. It is quite possible to mount tadpoles with the dorsal surface uppermost. A special method of technique is necessary to accomplish this last named end. Either Canada balsam or dammar is heated in an oven for some days until it will harden immediately upon cooling. Heated balsam or dammar of this character is then dropped upon slides. These drops should be of the proper width, to fill the space beneath a cover slip and to at the same time maintain the desired thickness. It is not the least difficult to get drops which may be 3 or 4 millimeters in depth.. They can be measured so as to give any proportion desired between breadth and depth. This can be done by heating the slide with the adherent drop over an alcohol lamp. It often chances that there are numerous small bubbles in such drops. These can be brought to the surface by placing the slides for some hours in a paraffin oven. They can then be removed by skimming the surface after heating, or in many cases they can be burst by application of a flame.

Large numbers of such slides can be prepared at a time and saved for subsequent use. They can be used, of course, for various mounts where it is desired to have the cover slip propped some distance above the slide, and in which immediate use is desirable.

Tadpoles, or other objects are mounted in the drop in the following manner: First, the drop is heated over a flame, being held there a moment or so inverted above it; then with a scalpel, previously dipped in a solvent such as xylol, a groove is made in it. This can be formed to suit the size and shape of the object to be mounted. The object is then placed in it—the sides of the groove pressed into contact with it in such a way as to hold it in the desired position. Then the space about the object is filled with a drop of soft balsam, and a cover slip, after being previously heated, is pressed down gently but firmly upon the surface of the drop. Care must be taken to prevent the inclusion of bubbles at this stage. A little practice will enable one to do this without difficulty.

The preparation should be laid aside for a week or more, and then examined. In some instances it will be found that the

objects have shifted somewhat in position. They can be adjusted by simply pushing the cover slip toward one or the other side. In this way the finest adjustments in position can be made. Objects can be brought from a very decided inclination to an upright position.

Preparations of tadpoles made in this manner are especially instructive in sowing the ventricles of the brain, the eye, the developing semi-circular canals of the ear, and the different portions of the alimentary tract. It is, of course, possible to make any desired dissections of embryos, and to mount them in this fashion. I found that preparations of the pronephros, mesonephros, and developing gonads can be handled very nicely in this manner. This use of hard balsam for mounting is not radically new, but is capable of much wider use than has ever been made of it.